Synthesis of [2'-H]-Ribonucleosides

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New syntheses of C(2')-deuterated ribonucleosides have been accomplished starting either from 3,5-di-O-benzyl-1-O-methyl-α,β-d-ribofuranose (1b) or 2,3-O-isopropylidene-α-ribose (14), with >97 atom-% D incorporation in both cases. The former is suited to the demands of multiple-site deuteration or uniform 13C/multiple 2H double labeling of the ribofuranose moiety, whereas the latter is particularly appropriate for single-site 2H labeling for mechanistic studies of enzyme reactions.

1. Introduction. – Various physico-chemical techniques have been exploited in order to correlate different structural motifs of oligo-DNA or oligo-RNA to their specific biological functions. Amongst these, NMR spectroscopy is one of the most powerful tools. The importance of NMR spectroscopy lies in its ability to provide the desired structural information under quasi-physiological conditions. However, with growing chain length, the usefulness of NMR spectroscopy becomes restricted due to the increasing spectral overlap [1], intrinsic line broadening arising from decreased T2 relaxation [1], decreased sensitivity caused by slower tumbling rate [1], and the spin diffusion that prevents accurate NOE volume measurements [2].

To tackle these problems, different isotope-labeling techniques have been reported so far in the literature. Although either chemical or enzymatic introduction of 13C/15N labels in the form of labeled monomers [3] into an oligo-RNA or oligo-DNA substantially reduces the spectral overlap in heteronuclear multidimensional spectra [4–6], the technique might be problematic for long oligomers for the following reasons: i) 13C labeling decreases proton T2 relaxation time, which, in turn, decreases the sensitivity of homonuclear J correlation techniques, ii) the syntheses of uniformly 13C-labeled 2'-deoxyribonucleosides is less elaborated [7–10] compared to the RNA counterparts [11–13], iii) 13C-signal broadening and signal loss due to long pulse sequences [14], iv) any enzymatic syntheses of labeled RNA [11–13] or DNA [7–10] do not allow the labeling of a particular segment of interest except ligation of labeled and natural stretches [15].

The early idea of using D labeling in NMR studies on oligonucleotides was based primarily on suppressing part(s) of the 1H-NMR spectrum [16]. However, it was soon found that site-specific or complete substitution of D for H in the sugar residues of nucleosides [17] and the sequence-specific incorporation of these specifically deuterated units into an oligomer not only simplifies spectral overcrowding, but enhances resolution and sensitivity, as well as efficiently reduces the deleterious effect of
relaxation. The cumulative effect is the facilitation of the structure elucidation as it has been demonstrated for the *Uppsala NMR window* concept [18].

When introduced into uniformly $^{13}$C-labeled nucleoside blocks, D labels have a positive effect on preserving the $^{13}$C magnetization during the long heteronuclear pulse trains in NMR experiments with uniformly $^{13}$C-labeled RNAs [19]. Since the primary source of $^{13}$C labels in the chemical synthesis of nucleosides with uniformly $^{13}$C-labeled pentofuranose moiety is $\text{D-}[^{13}\text{C}]_{6}$glucose, the need for D-glucose-based deuteration methods is fairly justified.

Besides NMR studies, investigations of the mechanisms of enzyme reactions have posed a further great demand for specifically deuterated nucleosides [17a][20]. Additional need for these labeled molecules might arise from studying the possible biological effect of the D substitution [21], which is an unexplored field for nucleosides and its derivatives.

The D incorporation into ribonucleosides according to an oxidation–reduction–inversion sequence at both nucleoside and sugar levels involves various methods, which differ in orthogonality of the protecting groups as well as in the type of the oxidation and reducing agents used [17a]. For example, the free C(2′)–OH of a 3′,5′-bis-O-protected ribonucleoside (by either (tert-butyl)dimethylsilyl (TBDMS) [22a,c,e] or 1,1,3,3-tetraisopropyldisiloxan-1,3-diyl group (TPDS) [18i][22b,e]) is oxidized with CrO$_3$/pyridine/Ac$_2$O [18i][22a,b,e] or DMSO/Ac$_2$O [22a] or DMSO/oxalyl chloride [18i][22d] or Dess–Martin reagent to give the corresponding C(2′)-oxo nucleoside [22c][22e]. The subsequent reduction with LiAlD$_4$ [22a] or NaBD$_4$ [18i][22a][22c] gives predominantly the arabino-diastereoisomer [18i][22a,c,d] owing to the preferential $\alpha$-attack over the $\beta$-attack; the exact ratio of arabino vs ribo depends, however, on the nature of the nucleobase. Subsequently, the inversion of the C(2′)–OH with arabino-configuration affords the required protected [2′-2H$_1$]ribonucleoside [18i][22c].

This approach requires the careful choice of the protection for the C(3′)–OH and C(5′)–OH groups. For example, the partial loss of the TPDS group during reduction of an adenosine derivative [22b] complicated its usefulness for the preparation of the target deuteronucleoside. Remarkable stereocontrol by the free C(5′)–OH group has been recently reported for 3′-O-TBDMS-2′-oxoadenosine and for a corresponding base-modified analog, when the reducing agent sodium triacetoxyborodeuteride [22c] is used to orchestrate a deuteride ion delivery from the $\beta$-face to give the ribo-product with excellent stereocontrol (ca. 99%). However, the utility of this method for the labeling of other nucleosides has not yet been corroborated. The 2′-2H labeling of nucleosides by the above oxidation–reduction sequence [22b] is further complicated by the fact that it gives an intractable mixture of products for guanosine derivatives. For the above reasons, clearly, D incorporation at C(2) at the sugar level, followed by glycosylation to give ribonucleoside, is an attractive alternative procedure. The oxidation of benzyl 3,4-O-isopropylidene-β-ν-arabinopyranoside by CrO$_3$/Ac$_2$O/pyridine, followed by reduction with LAD, furnished the [2′-2H$_1$]ribopyranoside derivative with high stereoselectivity (> 90%) [18i][22b]. A similar transformation commencing with Dess–Martin oxidation of 1,3,5-tri-O-benzoyl-α-D-ribofuranose [23a], followed by reduction with NaBD$_4$ in presence of CeCl$_3$, resulted in excellent stereoselectivity but gave relatively poor D enrichment (92–94 atom-% $^2$H, which is considered to be
insufficient for high-resolution NMR studies because of the interference by the background residual proton resonance lines). The instability of the Bz protecting groups during the reduction is an additional disadvantage of this methodology [23a]. A further consideration for the incorporation of D at the nucleoside level especially for the NMR studies is that the protecting-group manipulations for the D labeling are not the same as for the preparation of the phosphoramidite building blocks for oligo-RNA synthesis. Performing all protection—removal—reprotection steps with the individual nucleosides can substantially diminish the overall yield as compared to the preparation of the labeled sugar precursor. Hence, there is a good need for efficient alternative syntheses, which can overcome most of the above disadvantages. In addition, the new methodology should also be useful and practical for the multigram-scale preparation of the deuterionucleosides for relatively large-scale oligo-RNA synthesis for NMR work.

In our synthesis 2′-deoxy[2′(R/S),3′,5′(R/S)-2H₃]ribonucleosides, we introduced the third D at C(2′) through the reduction of the C(2′)-O sugar resulting from the oxidation of methyl 3,5-di-O-benzyl-α-β-[3,5(R/S)-2H₃]ribofuranoside (1a; Scheme 1) to give a mixture of predominantly arabino- and minor ribo-diastereoisomers 2a (ca. 7:3 as estimated by ¹H-NMR) [18i]. Similar stereoselectivity is exploited for the synthesis of β-D-mannosides through the reduction of β-D-glycoside-2-uloses [24] having 3-O-allyl or 3-O-benzyl groups vicinal to the C=O function. In the subsequent steps, the Bn groups of 2a were removed, and the resulting methyl [2,3,5(R/S)-2H₃]arabinofuranoside was separated from the corresponding ribo-component of 2a on a Dowex OH⁻ column, converted to the corresponding benzyl 3,4-O-isopropylidene-β-D-arabinopyranoside, and deuterated at C(2) according to our oxidation—reduction procedure discussed earlier [22b]. This long sequence of reactions (ten steps from 2 to 9) decreased the yield of the labeled methyl ribofuranoside substantially (overall yield of ca. 17% in ten steps), which is unacceptable for a building block to be used for milligram-scale solid-phase synthesis of large oligo-DNA (>20mer) with one or two NMR window(s) in the molecule for detailed solution-structure elucidation [18a,e,j].

We argued that if, upon appropriate protection of the C(2′)-OH, the mixture of the methyl 3,5-di-O-benzyl [2′-H₃]arabinos- and [2′-H₃]ribofuranosides 2b (arabinolribo 7:3 by NMR) could be successfully separated, and then the inversion of the arabinodiastereoisomer to ribo-analog could be achieved, it would constitute a much shorter and practical synthetic protocol for the D labeling at C(2′) of ribonucleosides. At this point, we also considered, as an alternative, the scale-up of the single-step D/H exchange reaction at C(2) (>97 atom-% ²H) of 2,3-O-isopropylidene-α-β-ribofuranose [23b] in a mixture of 1,4-dioxane/Et₃N/THF/H₂O at a reflux temperature. In this paper, the synthetic details of these two alternative chemical syntheses of [2′-²H₃]ribo-nucleosides are reported.

2. Results and Discussion. – For the synthesis of the required nucleosides, we wanted to take the advantage of a 2-O-acyl protection of the sugar precursor as in 11 (Scheme 1) during the glycosylation step, since this gives exclusively the β-anomer. Also, due to the known difficulties [25] to remove the Bn protecting groups of a nucleoside (specially with pyrimidine aglycones), a reasonable demand was to remove these groups still at the sugar level (i.e., 8 → 9). It is necessary to stress that the use of
4-methoxybenzyl instead of Bn protection might overcome the pyrimidine-reduction problem, since it involves a Ce(III)/Ce(IV)-promoted oxidation step [26], but its usefulness is yet to be documented in nucleoside synthesis.

The first method introduces D to C(2) at the sugar level starting from 1,2:5,6-di-O-isopropylidene-glucose, from which the starting 3,5-O-protected methyl ribofuranoside derivative 1b (Scheme 1) is easily available in large quantity (15 g) in six steps [27]. Compound 1b was subjected to Swern oxidation [28] with oxalyl chloride and DMSO in dry CH2Cl2 at 0 °C to 70 °C. The disappearance of the strong singlet at δ 3.32 for the anomeric Me group in the 1H-NMR spectrum for the α-anomer of 1b confirmed that the oxidation was complete (the same signal at δ 3.48 for the β-anomer of 1b overlaps with other signals in the 1H-NMR spectrum of the ketone). Reduction with LAD in dry Et2O afforded a diastereomeric C(2)-deuterated mixture 2b composed of arabinono- and ribofuranosides in a ratio of ca. 7:3 (64%), as evidenced by 1H-NMR. The arabinono-configuration in the main component is supported by the large upfield shift of C(1) (from δ 108.4 to 102.5). This mixture was successfully separated after 4-toluoylation at C(2)−OH to afford methyl 3,5-di-O-benzyl-2-O-(4-toluoyl)-α-[2-2H1]ribofuranoside (3) (26%) and methyl 3,5-di-O-benzyl-2-O-(4-toluoyl)-β-[2-2H1]arabinofuranoside (4) (62%).

The composition of the starting C(1) epimeric mixture (β/α ca. 7:3) indicated again [18i] that the arabinono-derivative might be formed diastereospecifically from the β-

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Scheme 1

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i) Oxalyl chloride, DMSO in CH2Cl2, 0 °C to 70 °C; ii) LiAlD4 in dry Et2O, r.t.; iii) TolCl, pyridine, r.t.; iv) NH3 in MeOH, r.t.; v) Tf2O, DMAP, Py, CH2Cl2, 0 °C, 3 h; vi) Cesium propanoate, DMF, r.t.; vii) Pd/C, H2 in EtOH, r.t.; viii) Ac2O, AcOH, conc. H2SO4, CH2Cl2, 0 °C, 15 min. ix) Persilylated nucleobase, TMSOTf, CH2Cl2 or toluene (12b), heating. Ac = Acetyl; Bn = benzyl; Bz = benzoyl; DMAP = 4-(dimethylamino)pyridine; Dpc = diphenylcarbamoyl; Pr = propanoyl; TMSOTf = trimethylsilyl trifluoromethanesulfonate; Tol = 4-toluoyl (=4-methylbenzoyl); A = adenin-9-yl; C = cytidin-1-yl; G = guanin-9-yl; U = uracil-1-yl.
anomer of 1b, whereas the ribo-compound arises from the α-anomer, i.e., the configuration of the anomeric MeO group controls the diastereoselectivity by specifically dictating the delivery of the deuteride ion from either α or β face. To gain deeper insight into the stereochemical course of the reduction of the 2-oxo compound diastereoisomer mixture of 1b was 2-O-(4-toluoyl)-protected, and the pure anomers were separated. The 4-toluoyl group was removed, and the β-anomer of 1b was subjected to Dess–Martin oxidation [17b][29]. Reduction of the ketone afforded a mixture containing two compounds in a ratio of ca. 91:9. The whole mixture was again 4-toluoylated, and the two compounds were separated by short-column chromatography (toluene/CH2Cl2 70:30 (v/v) with MeOH gradient). The separated major component proved to be the arabinoside 4, whereas the minor compound was identical, by 1H-NMR, to the methyl 3,5-di-O-benzyl-2-O-(4-toluoyl)-α-ribofuranoside except for bearing D at C(2). Similar oxidation-reduction steps carried out with the α-anomer gave ca. 4% of methyl 3,5-di-O-benzyl-α-arabinofuranoside as judged from the analysis of the 1H-NMR spectrum of the crude mixture in comparison with the literature data [30]. This reveals that the reduction is proceeding with a high diastereoselectivity.

Compound 3 can be deprotected to the α-anomer of 9 (Scheme 1) to improve the overall yield of the nucleoside precursor 11, as it has been shown in the preparation of [2',3',4',5',5'-H1]ribonucleosides [17b] but, in this study, it was used for exploring the use of the MeO group at the anomeric center of the sugar for the glycosylation step. However, a SnCl4-catalyzed synthesis of [2'-H1]ribothymidine and the subsequent deprotection proceeded with moderate yields (66 and 75%, resp.), hence this approach was abandoned. As mentioned above, the goal of this work required the inversion of configuration at C(2) of the arabino-compound 4 to give the ribo-counterpart 7. To achieve this the 2-O-Tol group of compound 4 was removed by methanolic ammonia at room temperature to afford arabinoside 5 (95%), followed by the conversion to the 2-O-triflate derivative 6 (89%) upon treatment with 4-(dimethylamino)pyridine, pyridine, and trifluoroacetic anhydride in dry CH2Cl2. In the absence of H–C(2), the presence of Tf moiety is corroborated by the appearance of a quadruplet 13C signal at δ 118.4 exhibiting the large J(C,F) coupling. Subsequently, compound 6 was converted to the 2-O-propanoyl derivative 7 (73%) by displacement with cesium propanoate in DMF at room temperature under an inert atmosphere [31]. The upfield shift of the peak from δ 4.99 to 4.87 for the corresponding anomeric proton H–C(1) proved that the reaction proceeded with an inversion of the configuration. Also, the change of the appropriate 13C chemical-shift order for C(3) and C(4) (C(3) (δ 80.8) > C(4) (δ 79.7) for the arabino-compound 6 to C(4) (δ 80.3) > C(3) (δ 77.7) for the ribo compound 7), together with the large downfield shift of C(1) (Δδ 5.9), corroborated the ribo-configuration at C(2). An attempt to convert the deuterated 2-O-propanoyl-ribofuranoside 7 to the 1-O-acetyl derivative via treatment with a Ac2O, AcOH, and concentrated H2SO4 mixture in CH2Cl2 in order to achieve higher coupling yields with the silylated nucleobases failed in our hand. This might be attributed to the presence of the two Bn groups, which are most probably unstable in the strongly acidic medium. Therefore, the 2-O-propanoyl group was cleaved upon treatment with methanolic ammonia to furnish compound 8 (98%), followed by the removal of the Bn groups in a catalytic hydrogenation process over 10% Pd/C in EtOH to afford the C(2)-deuterated...
methyl ribofuranoside 9 (98%). The spectral data were compared to those of its natural counterpart. The presence of a singlet at \( \delta 4.83 \) for H–C(1) and a doublet at \( \delta 4.1 \) (J(3,4) = 6.9 Hz) for H–C(3) obviated that more than 97 atom-% isotope incorporation has indeed taken place at C(2). Also, a low-intensity triplet (arising from the distribution of signal intensity and longer relaxation) was observed at \( \delta 73.6 \) in the \(^1\text{H}\)-decoupled \(^{13}\text{C}\)-NMR spectrum as a result of C,D coupling. Subsequently, compound 9 was 4-toluoylated to afford compound 10 (98%), followed by a treatment with a mixture of Ac₂O, AcOH, and concentrated H₂SO₄ in CH₂Cl₂ [18i] to obtain the required 1-O-acetyl-2,3,5-tri-O-(4-toluoyl)-α/β-D-[2-\(^2\text{H}\)]-ribofuranose (11) (99%). From this anomeric mixture, the β-anomer was crystallized from MeOH to give an analytical sample. When its \(^1\text{H}\)-NMR spectrum is compared to the spectrum of the natural counterpart (Fig. 1a and b), the lack of H–C(2) signal at \( \delta 5.75 \) and the appearance of the H–C(3) signal as a doublet at \( \delta 5.86 \) establish the >97 atom-% D incorporation as measured by integrating the residual H–C(2) signal. The specific optical-rotation measurement also established the identity of this compound (specific optical rotation for β-anomer of 11: +62, for authentic sample: +63).

The second method introduces the D label at C(2) starting from D-ribose. It has been reported from our laboratory [23b] that, on small scale (1 mmol), it is possible to obtain >97 atom-% diastereospecific exchange of the H at C(2) of 2,3-O-isopropylidene-ribose to D upon equilibration in dioxane/THF/Et₃N/2H₂O at elevated temperature. We herein report on specific conditions optimized for scaling up this exchange reaction to a level suitable for production of nucleosides for oligomer synthesis (ca. 22 mmol), which, upon several steps of synthetic manipulations, gave the 4-toluoylated ribose derivative 11 to be used for subsequent coupling with nucleobases (Scheme 2). The starting 2,3-O-acetonide 14 was prepared in a protic-acid-catalyzed reaction of D-ribose with acetone [32] in 78% yield. For the multigram-scale exchange of the ribofuranose 14, it was dissolved in ca. 3.5 ml/mmol of the mixture of dioxane/THF/Et₃N/2H₂O, instead of 20 ml/mmol [23b], and the \(^2\text{H}\)₂O amount was reduced from 4.6 ml/mmol [23b] to 0.73 ml/mmol, and the mixture was then heated at ca. 90°C for 5 days. The reaction proceeded without formation of detectable amount of side products to furnish the deuterated sugar 15 (99% yield). The deuteration level was found to be >97 atom-% as determined from the \(^1\text{H}\)-NMR spectrum. The deuterated ribose derivative 15 was converted to the labeled methyl ribofuranoside 16 (99%) by deprotection of the isopropylidene group in 80% aqueous AcOH, followed by glycosylation in MeOH in the presence of catalytic amount of concentrated H₂SO₄.

![Scheme 2](image)

i) Dioxane/THF/Et₃N/D₂O (24:24:12:16 ml), 90°C, 5 d. ii) 80% aq. AcOH, 90°C, 24 h. iii) MeOH, conc. H₂SO₄, 4°C, 12 h. iv) TolCl, Py, r.t. Tol = 4-Toluoyl.
Fig. 1. Expanded regions of the 270-MHz 1D-1H NMR spectra of 1-O-acetyl-2,3,5-tris-O-(4-toluoyl)-β-D-[2-2H]ribose (11f) (a) and its natural-abundance counterpart (b), 2,2',3',5'-tris-O-(4-toluoyl)-[2'-2H]uridine (12a) (c) and its natural-abundance counterpart (d), N2-acetyl-O6-(diphenylcarbamoyl)-2,2',3',5'-tris-O-(4-toluoyl)[2'-2H]guanosine (12b) (e) and its natural-abundance counterpart (f).
This ribose derivative 16 was 4-toluoylated by 4-methylbenzoyl chloride in dry pyridine to give the fully protected 17 (93%), which was further converted to 11 by the acetylation procedure as described for 10 [18i].

The coupling reactions with the persilylated uracil, N2-acetyl-O6-(diphenylcarbamoyl)guanine, N6-benzoyladenine, and N4-benzoylcytosine nucleobases were carried out according to well-established methods [33] to give the fully protected 17 (93%), which was further converted to 11 by the acetylation procedure as described for 10 [18i].

The coupling reactions with the persilylated uracil, N2-acetyl-O6-(diphenylcarbamoyl)guanine, N6-benzoyladenine, and N4-benzoylcytosine nucleobases were carried out according to well-established methods [33] to give the fully protected C(2)-deuterated nucleosides 12a–12d (88, 73, 85, and 70%, resp.). Comparison of their 1H-NMR spectra with those of the corresponding protected natural nucleosides (Fig. 1, c–f, and Fig. 2, a–d) evidences that no D-exchange reaction has taken place during the coupling process. The protecting groups were removed by treatment with NH3 in MeOH to produce the target [2/C39-2H1]-ribonucleosides 13a–13d (99, 85, 82, and 90%, resp.). The purity and the high-level isotope enrichment of these compounds are evidenced again upon comparison of their 1H-NMR spectra with those of authentic natural counterparts (Fig. 1, c–f, and Fig. 2, a–d, and Exper. Part). The identity of the nucleosides 13a–13d was further corroborated by high-resolution mass spectrometry, IR spectroscopy, as well as by optical-rotation measurements (see Exper. Part for details). It is interesting to note herein that, in the IR spectra of these monodeuterated carbohydrate and nucleoside analogues, no absorptions were observed in the region of the C–D stretching (2300–2000 cm−1) [34], even when films of the neat compounds were used as samples.

3. Conclusions. – Two new syntheses of C(2′)-deuterated ribonucleosides have been devised. Starting from 1-O-methyl-3,5-di-O-benzyl-α/β-D-ribofuranose (1), it has been found that i) Swern oxidation is suitable for complete oxidation of the C(2)−OH as a cheaper alternative to the Dess–Martin periodinane oxidation; ii) the reduction proceeds with high diastereoselectivity governed by the anomeric MeO group; iii) the present method substantially improves the overall yield from arabino-2 to ribo-9 (from 17 to 36%) as compared to the previous C(2)-deuterations [18i][22b] through benzyl arabinopyranoside; iv) the method is fully compatible with sequential multiple D incorporation (>97 atom-%) and with the ul-13C-labeled d-glucose-based 2H/13C double labeling. The second method uses 2,3-O-isopropylidene-d-ribose (14) as starting material, and a large-scale exchange process gives >97 atom-% D incorporation in a single equilibration step. Because of its simplicity, it is well-suited for C(2′) single-site labeling for exploring mechanistic aspects of enzyme reactions.

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Experimental Part

General. CH2Cl2 and 1,2-dichloroethane were refluxed with P2O5, followed by distillation under N2 and kept over molecular sieves (3 Å). Pyridine was refluxed on CaH2 overnight, followed by distillation and stored over molecular sieves (3 Å). The chromatographic separations were performed on Merck G60 silica gel. TLC was performed on Merck pre-coated silica gel 60 F254 glass backed plates in following systems: A) toluene/CH2Cl2/MeOH 7:3:0.1 (v/v/v), B) AcOEt/cyclohexane 1:1 (v/v), C) AcOEt/cyclohexane 70:30 (v/v), D) AcOEt/PrOH/H2O 30:18:6 (v/v/v), E) AcOEt, F) MeOH/CH2Cl2 10:90 (v/v), G) petroleum ether/AcOEt 8:2. 1H-NMR Spectra: at 270.17 MHz, with TMS or MeCN (for D2O solns., set at 2.0 ppm) as internal
Fig. 2. Expanded regions of the 270-MHz 1D-1H-NMR spectra of N°-benzoyl-2',3',5',-tris-O-(4-toluoyl)]2'-'H₂adenosine (12c) (a) and its natural-abundance counterpart (b), N°-benzoyl-2',3',5'-tris-O-(4-toluoyl)]2'-H₂cytidine (12d) (c) and its natural-abundance counterpart (d), [2'-H₂]uridine (13a) (e) and its natural-abundance counterpart (f).
Fig. 3. Expanded regions of the 270-MHz 1D-1H NMR spectra of [2-2H]guanosine (13b) (a) and its natural-abundance counterpart (b), [2-2H]adenosine (13c) (c) and its natural-abundance counterpart (d), [2-2H]cytidine (13d) (e) and its natural-abundance counterpart (f).
standards. $^{13}$C-NMR Spectra: at 67.9 MHz, with the central peak of CDCl$_3$ (76.9 ppm), (D$_6$)DMSO (39.6 ppm), or MeCN (1.3 ppm) as references. Chemical shifts ($\delta$) in ppm, coupling constants in Hz; signal assignments for each compound were achieved by 2D-NMR experiments ($^1$H-H-COSY, $^1$H-$^1$C-HETCOR, COLOC) with standard software. $^{13}$C Multiplicities and overlapping quaternary signals were identified by APT experiments. Assignments were also corroborated with comparison literature data. 

Data of 3. $R_g$ (G) 0.69, $[\alpha]_D^{20} = -98$ (c = 0.67, CHCl$_3$). IR (neat): 3459, 3084, 3060, 3030, 2920, 2859, 1494, 1452, 1362, 1308, 1202, 1156, 1095, 1049, 1029, 1010, 910, 852, 819. $^1$H-NMR (CDCl$_3$): 7.34 - 7.25 (m, 2 PhCH$_2$); 4.86 (s, 1 H - C(3)); 4.77 - 4.52 (m, 2 PhCH$_2$); 4.29 (d, $J(3,4) = 5.4$, H - C(3)); 3.55 - 3.32 (m, 4 PhCH$_2$); 2.42 (s, Me$_2$CH$_3$). $^{13}$C-NMR (CDCl$_3$): 168.5 (C-O - Tol); 145.9, 137.9, 137.6, 129.8, 129.0, 128.3, 127.7, 127.6, 127.6, 126.6 (PhCH$_2$ - Tol); 101.3 (C(1)); 81.3 (C(3)); 79.6 (C(4)); 73.3 (PhCH$_3$); 72.0 (C(5)); 71.9 (PhCH$_3$); 71.8 (C(5)); 71.7 (CH$_2$); 55.3 (MeO). HR-ESI-MS (pos.) 463.2109 (M$^+$, C$_{20}$H$_{23}$O$_3$H$_2$O$^+$); calc. 463.2106.

Methyl 3,5-Di-0-benzyl-2-O-4-(methylbenzyl)-3-O-(2-H$_2$)ribofuranoside (5). Methanolic ammonia was added to 4 (60 g, 12.94 mmol), and the solution was stirred at r.t. for 2 d. Removal of solvent and purification by silica-gel CC gave 5 (4.23 g, 95%). $R_g$ (G) 0.29, $[\alpha]_D^{20} = -49$ (c = 0.32, CHCl$_3$). IR (neat): 3459, 3084, 3060, 3030, 2920, 2859, 1494, 1452, 1362, 1308, 1202, 1156, 1095, 1049, 1029, 1010, 910, 852, 819. $^1$H-NMR (CDCl$_3$): 7.34 - 7.25 (m, 2 PhCH$_2$); 4.86 (s, 1 H - C(3)); 4.77 - 4.52 (m, 2 PhCH$_2$); 4.29 (d, $J(3,4) = 5.4$, H - C(3)); 3.55 - 3.32 (m, 4 PhCH$_2$); 2.42 (s, Me$_2$CH$_3$). $^{13}$C-NMR (CDCl$_3$): 137.9, 137.8, 128.3, 127.6, 127.6, 126.6 (PhCH$_2$ - Tol); 101.3 (C(1)); 81.3 (C(3)); 79.6 (C(4)); 73.3 (PhCH$_3$); 72.0 (C(5)); 71.98 (PhCH$_3$); 55.2 (MeO). HR-ESI-MS (pos.) 463.2109 (M$^+$, C$_{20}$H$_{23}$O$_3$H$_2$O$^+$); calc. 463.2106.

Methyl 3,5-Di-0-benzyl-2-O-3-fluoromethylthiofuranon-3-O-(2-H$_2$)ribofuranoside (6). Compound 5 (4.18 g, 12.10 mmol) was co-evaporated with dry pyridine, and it was dissolved in dry CH$_2$Cl$_2$ (90 ml), followed by the addition of DMAP (5.18 g, 42.4 mmol) and DMSO$_2$. The reaction mixture was stirred for 2 h, followed by the addition of DMAP (5.18 g, 42.4 mmol) and DMSO$_2$. The mixture was cooled to 0$^\circ$C and THF (2.8 ml, 16.96 mmol) was added dropwise, and the resulting mixture was stirred at the same temp. for 3 h. The mixture was poured into cold sat. NaHCO$_3$ soln. The org. layer was separated, and the H$_2$O phase was extracted with CH$_2$Cl$_2$. The combined org. extract was dried (MgSO$_4$) and concentrated. The residue was purified by column chromatography (CC) to yield 6 (7.2 g, 64%). $R_g$ (G) 0.59, $[\alpha]_D^{20} = -64$ (c = 0.74, CHCl$_3$). HR-ESI-MS (CDCl$_3$): 7.35 - 7.20 (m, 2 PhCH$_2$); 4.99 (s, 1 H - C(1)); 4.76 - 4.47 (m, 2 PhCH$_2$); 4.29 (d, $J(3,4) = 5.4$, H - C(3)); 4.17 - 4.11 (m, 1 H - C(4)); 3.58 - 3.44 (m, 4 PhCH$_2$); 3.38 (s, MeO). $^{13}$C-NMR (CDCl$_3$): 137.6, 136.8, 128.4, 128.3, 127.7, 127.6 (PhCH$_3$); 118.4
anomer was crystallized from MeOH as a white solid (1.87 g, 59%).

\[ 12a \]

soln. and separation on a silica-gel column gave 4 h. The volatile materials were evaporated, and residue was kept on an oil pump for 20 min. Compound (450 mg) in EtOH (40 ml) over 3 h at r.t. The reagent was filtered through hexamethyldisilazane (4.7 ml), and Me 3SiCl (0.5 ml) was added. The mixture was stirred at 120 °C for 2.5 h. IR (KBr): 3060, 3055, 2950, 2920, 1720, 1685, 1609, 1541, 1380, 1376, 1270, 1210, 1180, 1157, 1129.

Methyl 3,5-Di-0-benzyl-β-D-ribofuranoside (8). Compound 7 (2.7 g, 6.67 mmol) was treated with methanolic ammonia under stirring at r.t. for 30 h. Usual workup, followed by removal of solvent on a rotary evaporator and washing with H2O, gave chromatographically homogeneous compound 8 (2.3 g, 99%). Rf (G) 0.27. \[ \beta_0 \] = 29 (c = 0.71, CHCl3). IR (neat): 3459, 3082, 3060, 2959, 1494, 1452, 1432, 1204, 1158, 1085, 1063, 910, 873, 810. 1H-NMR (CDCl3): 7.38–7.46 (m, 2 PhCH3); 4.86 (s, H–C(1)); 4.57 (2 s, 2 PhCH3); 4.26–4.20 (m, H–C(4)); 4.07 (d, J(3,4) = 6.2, H–C(3)); 3.54 (d, J(4,5) = 6.3, CH5)); 3.31 (s, MeO). 13C-NMR (CDCl3): 138.0, 137.0, 128.5, 128.2, 127.5 (PhCH3); 106.2 (C(1)); 80.3 (C(4)); 71.7 (C(3)); 73.1, 72.9 (2 PhCH3); 71.1 (C(5)); 54.9 (CH3O); 27.3 (MeO); 8.9 (MeO). HR-ESI-MS (pos): 401.1955 (M+, C6H11HO23; calc: 401.1949).

Methyl 3,5-Di-0-benzyl-β-D-ribofuranoside (9). The Bn groups of 8 (2.1 g, 6.08 mmol) were cleaved with Pd/C–H2 (450 mg) in EtOH (40 ml) over 3 h at r.t. The reagent was filtered through Celite, and the filtrate was evaporated to dryness to afford 9 (980 mg, 98%). Rf (C) 0.58. \[ \beta_0 \] = 38 (c = 0.15, H2O). 1H-NMR (CDCl3): 4.83 (s, H–C(1)); 4.08 (d, J(3,4) = 6.8, H–C(3)); 3.98–3.92 (m, H–C(4)); 3.76–3.51 (dq, J(4,5) = 3.3, J(4,5) = 6.4, J(5,6) = 12.2, CH5)); 3.33 (s, MeO). 13C-NMR (CDCl3): 107.7 (C(1)); 82.7 (C(4)); 71.5 (C(5)); 71.4 (C(6)); 63. IR (KBr): 3060, 3036, 2959, 1494, 1452, 1432, 1204, 1158, 1085, 1063, 910, 873, 810. 1H-NMR (CDCl3): 7.38–7.46 (m, 2 PhCH3); 4.86 (s, H–C(1)); 4.57 (2 s, 2 PhCH3); 4.26–4.20 (m, H–C(4)); 4.07 (d, J(3,4) = 6.2, H–C(3)); 3.54 (d, J(4,5) = 5.3, CH5)); 3.31 (s, MeO). HR-ESI-MS (pos): 345.1692 (M+, C16H22O23; calc: 345.1687).

1-O-Acetyl-2,3,5-tris-O-(4-methylbenzoyl)-β-D-ribofuranoside (10). Compound 9 (980 mg, 5.93 mmol) was co-evaporated with dry pyridine twice and dissolved in the same solvent (50 ml). 4-Methoxybenzoyl chloride (2.6 ml, 19.52 mmol) was added dropwise in an ice-bath under stirring. The mixture was kept for 15 h allowing it to warm to r.t. Sat. aq. NaHCO3 soln. was added and stirred for 3 h. The compound was extracted with CH3Cl from H2O. Evaporation under reduced pressure gave chromatographically homogeneous 10 (2.97 g, 96%). Rf (C) 0.82. \[ \beta_0 \] = 75 (c = 0.17, CHCl3). 1H-NMR (CDCl3): 7.98–7.20 (m, 3 MeC6H4); 5.83 (d, J(3,4) = 6.8, H–C(3)); 5.13 (s, H–C(1)); 4.74–4.67 (m, H–C(4), H–C(5)); 4.52–4.45 (dd, H–C(5)); 3.40 (s, MeO); 2.40, 2.39, 2.36 (3s, 3 MeC6H4). 13C-NMR (CDCl3): 166.2, 165.3, 165.2 (3 C = O, Tol); 144.0, 143.9, 143.6, 129.7, 129.6, 129.0, 128.9, 126.9, 126.6, 126.2, 126.1 (2 Tol); 106.3 (C(1)); 79.0 (C(4)); 72.1 (C(3)); 64.5 (C(5)); 55.2 (MeO); 21.5 (Me, Tol). HR-ESI-MS (pos): 519.2004 (M+, C20H22O23; calc: 519.2004).
4.04 (m, 3 H, white foam). 1H-NMR (D2O): 7.78 (1111, 1092, 1019. 1H-NMR (CDCl3): 8.47 (br.
1091, 1019. 1H-NMR (CDCl3): 8.01 ± 7.84 (144.48 (Me
125.7 (Me
1083, 1045, 1012, 989, 865, 828, 778, 690. 1H-NMR ((D6)DMSO 50
differentiation, 3 days at r.t. After evaporation of MeOH, the residue was dissolved in H2O, and extracted
(125.7 (Me
1058, 1018, 979.1H-NMR (CDCl3): 8.10 (br.
1506, 1479, 1450, 1407, 1389, 1380, 1378, 1377, 1357, 1318, 1267, 1211, 1186, 1116, 1088, 1082, 1071, 1049, 1025, 960, 940, 930, 900, 870, 849, 829, 761. 1H-NMR (D2O): 7.78 (d, J=5.6) = 8.1, H – C(6); 5.83 (s, H – C(1)); 5.81 (d, H – C(5)); 4.15 (d, H – C(3)); 4.08 – 4.04 (m, H – C(4)); 3.69 (dd, J=5.4 = 2.9, J=5.8 = 12.8, J=5.4 = 4.3, CH3(5)); 13C-NMR (D2O): 166.0 (C(4)); 151.1 (C(2)); 141.7 (C(6)); 102.1 (C(5)); 89.2 (C(1)); 84.0 (C(4)); 69.2 (C(3)); 60.6 (C(5)). HR-ES-MS (pos.): 245.0759 (M+ C2H3H3NNO2; calc. 245.0758).
13HN1/guanosine (13b). Compound 12b (630 mg, 0.72 mmol) was deprotected by stirring in methanolic
ammonia (50 ml) and stirred at r.t. overnight. The sample for analysis was recrystallized from H2O. [α]D2 = −36 (c = 0.04, H2O) ([α]D2 for natural guanosine = −37). IR (KBr): 3420, 3320, 3150, 2929, 2748, 1683, 1625, 1592, 1531, 1480, 1408, 1360, 1350, 1241, 1172, 1083, 1045, 1012, 910, 890, 865, 828, 778, 690. 1H-NMR ((D2)DMSO 50’: 8.00 (s, H – C(8)); 6.54 (br. x, NH2);
5.82 (s, H–C(1’)); 4.22 (d, J(3’,4’) = 3.6, H–C(3’)); 4.03–3.99 (m, H–C(4’)); 3.78–3.63 (ddd, J(5’,4’) = 3.9, J(5’,5’) = 11.8, J(5’,4’) = 3.9, CH(5’)). 13C-NMR (D2O): 157.3 (C(6)); 153.9 (C(2)); 151.8 (C(4)); 136.3 (C(8)); 117.1 (C(5’)); 86.8 (C(1’)); 85.6 (C(4’)); 70.7 (C(3’)); 61.7 (C(5’)). HR-ESI-MS (pos.): 284.0983 (M+, C9H10NO; calc. 284.0979).

[2'-2H2]Adenosine (13k). Nucleoside 13k (70 mg, 82%) was obtained as white powder after deprotection of 12 (52.3 mg, 0.32 mmol) in methanolic ammonia, [α]29D +5 (c = 0.17, H2O). [α]29D +15 (natural adenosine) = –60. IR (KBr): 3430, 3320, 3160, 2918, 1615, 1642, 1601, 1573, 1474, 1417, 1380, 1338, 1291, 1245, 1208, 1177, 1158, 1110, 1086, 1078, 1050, 1028, 960, 970, 880, 835, 791, 749. 1H-NMR (D2O): 8.21 (s, H–C(8)); 8.08 (s, H–C(2)); 5.95 (s, H–C(1’)); 4.34 (d, J(3’,4’) = 3.2, H–C(3’)); 4.23–4.19 (m, H–C(4’)); 3.88–3.73 (ddd, J(5’,4’) = 2.7, J(5’,5’) = 12.9, J(5’,4’) = 3.5, CH(5’)). 13C-NMR (D2O): 155.3 (C(6)); 152.2 (C(2)); 148.3 (C(4)); 140.6 (C(8)); 119.1 (C(5’)); 88.2 (C(1’)); 85.8 (C(4’)); 70.5 (C(3’)); 61.5 (C(5’)). HR-ESI-MS (pos.): 268.1036 (M+, C9H10NO; calc. 268.1030).

[2'-2H2]Cytidine (13d). Compound 12d (220 mg, 0.31 mmol) was stirred overnight in methanolic ammonia, followed by removal of MeOH. Dissolving the residue in H2O, and successive washings with CHCl3 (3 × ) and EtO afforded 13d (68.2 mg, 90%). [α]29D +32 (c = 0.08, H2O). 1H-NMR (natural cytidine) = +33. IR (KBr): 3340, 3200, 2920, 2715, 1640, 1600, 1521, 1483, 1398, 1370, 1282, 1242, 1206, 1152, 1120, 1082, 1040, 1025, 965, 882, 858, 780. 1H-NMR (D2O): 7.77 (d, J(5,6) = 7.6, H–C(6)); 5.98 (d, H–C(5)); 5.82 (s, H–C(4)); 4.13 (d, J(3,4) = 5.9, H–C(3)); 4.08–4.03 (m, H–C(4’)); 3.89–3.70 (ddd, J(5’,4’) = 3.0, J(5’,5’) = 12.7, J(5’,4’) = 4.3, CH(3’)). 13C-NMR (D2O): 166.1 (C(4’)); 157.5 (C(2)); 141.5 (C(6’)); 96.0 (C(5’)); 90.1 (C(1’)); 83.6 (C(4’)); 69.1 (C(5’)); 60.6 (C(3’)). HR-ESI-MS (pos.): 244.0922 (M+, C9H10NO; calc. 244.0918).

1,3-Di-O-propylidenyl-β-D-ribofuranose (14). 2,3-O-Isopropylidenyl-α-D-ribofuranose (15). 2,3-O-Isopropylidenyl-d-ribofuranose (16). Compound 15 (4.18 g, 22.0 mmol) was co-evaporated with D2O and dissolved in dioxane/THF/Et3N/D2O (24 : 24 : 12 : 16 ml (v/v/v/v)). The soln. was heated at 80 for 90 d, and evaporation gave 15 (4.18 g, 99%). Brown oil. Rf (F) 0.53. 1H-NMR (CDCl3); β-Anomer: 5.39 (s, H–C(1’)); 4.84 (s, H–C(3’)); 4.40–4.37 (m, H–C(4’)); 3.75–3.66 (m, CH(5’)); 1.49, 1.32 (2x, 2 Me); α-Anomer: 5.42 (s, 0.12 H, H–C(1’)); 4.74 (d, J(3,4) = 2.2, 0.12 H, H–C(4’)); 4.19–4.16 (m, 0.12 H, H–C(4’)); 3.75–3.66 (m, 0.24 H, CH(5’)); 1.58, 1.40 (2x, 0.72 H, 2 Me). 13C-NMR (CDCl3); β-Anomer: 111.8 (Me-C); 102.5 (C(1’)); 87.5 (C(4’)); 81.6 (C(3’)); 63.2 (C(5’)); 28.2, 24.6 (2 Me); α-Anomer: 113.8 (Me-C); 96.9 (C(3’)); 81.3 (C(5’)); 80.9 (C(3’)); 61.2 (C(5’)); 25.9, 24.6 (2 Me). HR-ESI-MS (pos.): 191.0077 (M+, C9H16O5; calc. 191.0094).

Methyl β-D-ribofuranoside (17). Compound 16 (3.58 g, 21.8 mmol) was co-evaporated with dry pyridine and dissolved in pyridine (150 ml). 4-Methylbenzyl chloride (9.6 ml, 72.6 mmol) was added in 5 portions at 0°, and the mixture was stirred overnight. It was poured into sat. aq. NaHCO3 soln. and stirred for 2 h, followed by extraction with CHCl3. Solvents were evaporated, and the residue was co-evaporated with toluene. The oily residue was subjected to CC to afford 17 (10.67 g, 99%). Rf (C) 0.81. 1H-NMR (CDCl3): 8.05–7.99 (m, 15.6 H, MeC6H4 (α + β)); 5.83 (d, J(3,4) = 6.7, H–C(3) (α)); 5.68 (d, J(3,4) = 3.3, 0.3 H, H–C(3) (α)); 5.37 (s, 0.3 H, H–C(1) (α)); 5.13 (s, H–C(1) (β)); 4.74–4.45 (m, 3.9 H, H–C(4) (α), H–C(4) (β), CH(5) (α), CH(5) (β)); 3.47 (s, 0.9 H, MeO (α)); 3.40 (s, MeO (β)); 2.46–2.36 (m, 11.7 H, Me (α + β)). 13C-NMR (CDCl3); β-Anomer: 166.2, 165.3, 165.2 (C = O); 144.1, 144.0, 143.6, 129.7, 129.6, 129.4, 128.9, 127.0, 126.9, 126.2 (MeC6H4); 106.3 (C(1’)); 79.1 (C(4’)); 72.1 (C(3’)); 64.5 (C(5’)); 55.2 (MeO); 21.5 (Me); α-Anomer: 166.1, 165.9, 165.4 (C = O); 145.4, 143.8, 130.5, 129.9, 129.0, 126.8, 126.4, 126.1 (MeC6H4); 101.8 (C(1’)); 79.5 (C(4’)); 70.5 (C(3’)); 63.8 (C(5’)); 55.6 (MeO); 21.7 (Me). HR-ESI-MS (pos.): 519.2010 (M+, C9H16O5; calc. 519.2004).


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